National Institute of Biomedical Imaging and Bioengineering

Molecular Footprints Step Up Drug Development

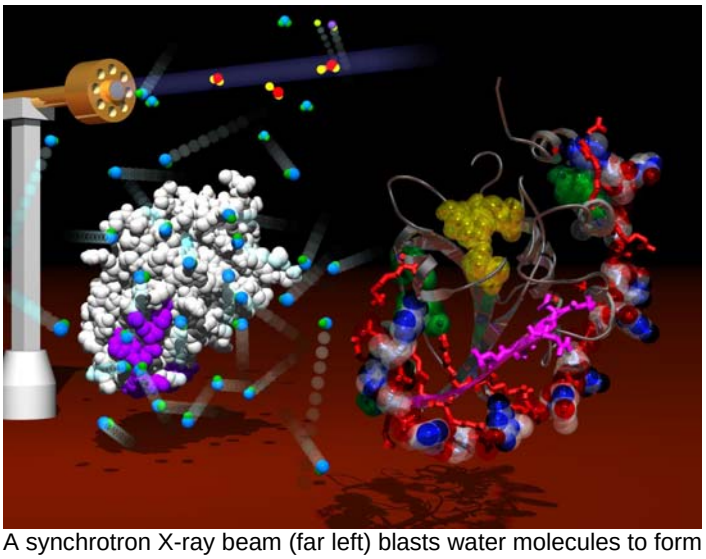
A state-of-the-art imaging study has detailed for the first time how viral DNA interacts with an enzyme crucial for viral replication. Armed with the new information, scientists have already started searching for drugs that might treat infections by disrupting that interaction.

The researchers used a method known as synchrotron X-ray footprinting to learn exactly how viral DNA binds to and activates the enzyme. Enzymes are biological catalysts that regulate a variety of processes within living cells. With X-ray footprinting, the scientists could pinpoint contact points between the DNA molecule and the enzyme molecule. Three-dimensional computer simulations then revealed that the DNA wraps itself over more than half the viral enzyme's surface, providing ample targets for possible antiviral drugs.

Unzipping Enzymes

This extensive binding appears to open up two regions of the enzyme molecule, exposing its active site and making the enzyme more potent, says Dr. Mark Chance, director of the Albert Einstein Center for Synchrotron Biosciences at the Albert Einstein College of Medicine. The DNA acts like a tight elastic strap stretched across the two regions, prying them apart like a clamshell.

Dr. Chance and his colleagues studied the enzyme known as adenovirus proteinase (AVP), derived from the human adenovirus. A culprit in respiratory, gastrointestinal, and eye infections, adenovirus causes colds and pinkeye and can sometimes lead to blindness. Because the AVP enzyme shares features with related enzymes from many other pathogens, including HIV, studies of AVP may aid drug development for a variety of infectious diseases.



A synchrotron X-ray beam (far left) blasts water molecules to form hydroxyl radicals. These react with chemical chains on an adenovirus protease molecule (left) to reveal, in the 3-D model at right, how adenoviral DNA (red) binds to and activates the AVP protease.
Image used with permission from the American Society for Biochemistry and Molecular Biology.

As a protease, or protein-cleaving enzyme, AVP breaks apart so-called scaffolding proteins that help the adenovirus to build new virus particles. Just as a newly constructed building cannot be used until workers remove the scaffolding, new adenovirus particles cannot mature and become infectious until AVP has dismantled their scaffolding proteins. But AVP must first be switched on, or activated, before it can tear down the scaffolding.

Recently, study coauthor Dr. Walter Mangel, a biologist at the Brookhaven National Laboratory, and his colleagues discovered that adenoviral DNA helps to activate AVP. That finding led them to propose that new drugs might hinder replication of the adenovirus by keeping the AVP protease and DNA apart. However, the scientists needed a better understanding of how AVP and DNA interact.

"Mangel came to us because he suspected that the DNA contacted the protease in some specific regions, but he didn't have hard evidence," Dr. Chance says. "At the time, the footprinting technology had not been perfected, so we thought this would be a good test molecule for us to examine."

Dr. Chance's center invented the synchrotron X-ray footprinting technique in the late 1990s. He and his colleagues continue to improve the technology under a five-year, $5.1 million grant awarded in 2003 by the National Institute of Biomedical Imaging and Bioengineering.

Visualizing Molecular Activity

Synchrotron X-ray footprinting takes place in a ring-shaped building called a synchrotron. The synchrotron accelerates electrons to produce high-intensity X-rays. Scientists shoot the synchrotron X-rays at water molecules to form hydroxyl radicals, which then interact with a protein or other molecule, cleaving it at key points. Portions of the molecule buried in folds or in regions bound to other molecules are protected from the hydroxyl radicals. The lack of breakage at those sites leaves "footprints" that show where molecular folds and chemical bonds occur.

The researchers used footprinting to identify points of contact between AVP and DNA. Three-dimensional computer modeling then visualized the molecular interaction based on those contact points. The 3-D image of the molecules after binding clearly shows the active site of the enzyme becoming more accessible. "Converting the footprinting data to a 3-D model raises the bar for our footprinting studies in the future," Dr. Chance says.

Dr. Chance's team continues to improve the footprinting imaging technology. The researchers are finding ways to visualize more massive biological entities, such as large macromolecular complexes. They are also expanding the number of chemical structures accessible to footprinting. "You want to see as much of the protein surface as possible," Dr. Chance says.

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Reference

Gupta S, Mangel WF, McGrath WJ, Perek JL, Lee DW, Takamoto K, Chance MR. DNA binding provides a molecular strap activating the adenovirus proteinase. Molecular & Cellular Proteomics 3:950-959, 2004.